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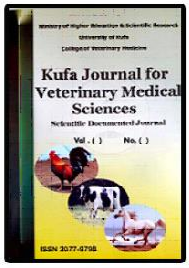

Gross and histopathological changes in liver and lung of cattle and sheep infected with hydatid cyst

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Abstract

The present study was conducted in the Al-Najaf slaughterhouse, during the period from November 2020 to March 2021, were collected 200 specimens of infected liver and lungs of sheep and cattle. The objective of the study is to investigate the number of hydatid cyst infections in cattle and sheep to investigate the macroscopic and microscopic characteristics, number and size of cysts in each lung and liver, comparison between the thickness of cysts wall in the liver of sheep and cattle infested with echinococcosis. The results of the macroscopic study showed the total number of cysts found in the liver of slaughtered sheep was 673 in different size groups while in the lung of slaughtered sheep, the total number of cysts was 216 cysts in different size groups. The examination of the lung of infected cattle during the period of our study revered no cystic lesions in the study while the total number of cysts found in the liver of slaughtered cattle was 158 in different group sizes, almost 50 % of the size of the hydatid cyst in the sheep liver is between 1-3 cm, while in cattle is between 3-5 cm. The microscopic investigation of the liver and lung specimens stained with hematoxylin and eosin stain and another stain was used in this study to evaluate the fibrous tissue proliferation via Masson's trichrome stain. showed there were features of chronic inflammation in hepatic tissue of infested sheep and cattle by cystic echinococcosis represented by marginal fibrous tissue layer surrounding the hydatid cyst, infiltration of mononuclear cells, eosinophils, neutrophils, macrophages, and fibrocytes. The main conclusions of the study revealed that there were significant differences between the thickness of germinativum, laminated and adventitial layers of hydatid cysts in different size groups, and no hydatid cysts were recognized in the lung of slaughtered cattle during the study period.

Keywords: cattle, gross, hydatid cysts, histopathological, sheep.

Introduction

Echinococcosis is a zoonotic disease, that caused by *Echinococcus granulosus (E. granulosus*); dogs and foxes are usually the final hosts; were transmitted to herbivorous e.g. (man, sheep, cattle camels, and pigs) which consider as intermediate host [1]. the fecal-oral main route of infection by consumption of the eggs that are released from the stool of the final host. The development of parasites occurs in the intermediate host organs include the lung or liver [2]. The echinococcosis (Metacestode stage or larval stage) has very slowly grown where 1 cm in size reaches by the 5th month [3]. In the final

stage of hydatid cyst development filled by about 15 quarts of fluid (hydatid fluid) while some of the cysts are sterile [4]. *E. granulosus* cysts of goats and sheep mostly infect the liver and lungs. Sheep has a hypersensitivity reaction, and there is no sudden death in sheep and other animals [5]. Cysts are frequently unilocular and multiple with liver and\or lung in cattle. The growth of Cysts is slow and may take long-life cysts. In the horses, the liver cyst may stay small and without clinical signs [6]. The clinical manifestations are noted in one case reported in cattle presented with large liver enlargement, liver dysfunction, raised levels of liver enzymes, intermittent pain, obstructive lung disease, emaciation, and eating disorder, the pathological effects depend on the location of the hydatid cyst, a ruptured cyst in the liver through the diaphragm can be caused by a bronchobiliary fistula or pleural effusion [7], [8] reported the hydatid cysts of the liver opened in the biliary duct in 25% of cases and verified that biliary, jaundice, and eosinophilia are features of rupture into the biliary tree. Other researchers [9] described a case with acute calculus cholecystitis because of a hydatid cyst in the liver that ruptured into the gall bladder, obstruction by daughter cyst in cystic duct .There's thin fibrous wall and some granulation. Deeply stained nuclei (pyknotic) of Kupffer cells, hepatocytes, walls of the blood vessels, and bile ducts are usually recognized with lymphocytopenia in the portal area with Kupffer cells. numerous karyolytic nuclei can also recognized [9]; [10]; [11] ; [12] [13] [14]. The distraction of portal area with an endothelial rupture that lining the hepatic portal vein and thickened arterial wall, also can be noticed[15].

Materials and Methods

Ethical approval:

According to Islamic law and regulations, which approved by the institutional committee of animal care and use (IACUC) manuscript no.3219 on February ,14, 2021.

Study design:

These animals were slaughtered. From November 2020 to March 2021, The specimens of slaughtered sheep and cattle were picked up from different regions of Al-Najaf province to Al-Najaf abattoir, for slaughtering. Firstly, the veterinarians' examined these animals to ensure their suitability to slaughtering and respect of general health. Few minutes after slaughtering the liver and lung were obtained to study their morphological and microscopically characteristics (Number of cysts in each organ, size of cysts in each organ by using vernix caliber, any abnormal changes in color, size, consistency of organ, any abnormal lesions in organ). The total number of specimens was 200 (50 for liver of cattle, 50 for liver of sheep and 50 for lung of sheep. tissue sections (1 cm X 1 cm) were collected and transported to the tissue processing unite. The collected specimens were used to study the histopathological changes using routine H&E staining, Masson's trichrome staining.

Statistical Data Analysis

Tests for normality were run on the wall thickness of the hydatid cysts in the liver and lung of sheep and cattle and different layers values using normality test. All data were analyzed by Tukey's Multiple Comparison Test one-way ANOVA (Graph pad Prism v.8), P-value was considered significant at <0.05 [16].

Results

Gross pathology

Macroscopic evaluation of liver and lung of slaughtered sheep and cattle in AL- Najaf abattoir among those 100 specimens of liver and lung from slaughtered sheep. Showed 673 cyst in liver of slaughtered sheep. Range from

one cm to five cm. The number of cysts in liver less than 1cm were 181 cysts (26.9%), 1-3 cm 323 cysts (47.76%), 3-5cm 127 cysts (18.87%) as in figure 1 and in more than 5 cm 42 cysts (6.24%) in table 1, while total number of cysts in lung of slaughtered sheep were 216 cysts, number of cysts less than 1cm were 48 (22.22%), 1-3 cm were 62 (28.7%), 3-5 cm were 77 (35.64%) as in figure 2 and in more than 5 cm were 29 (13.42%) in table 2.

Table 1. Numbers and percentages of hydatid cysts according to sizes in liver of sheep

Size	**1>cm**	**1-3cm**	**3-5cm**	**5<cm**
Number	181	323	127	42
Percentage *	26.90%	47.76%	18.87%	06.24%

Table 2. Number and percentage of hydatid cysts according to sizes in lung of slaughtered sheep

Size	**1>cm**	**1-3 cm**	**3-5 cm**	**5<cm**
Number	48	62	77	29
Percentage*	22.22%	28.70%	35.64%	13.42%

The examination of lung of slaughtered cattle during the period of our study revered that no cystic lesions in all collected specimens while the hepatic lesions have appeared prominently in the liver of slaughtered cattle with 158 cysts. the number of cysts with less than 1 cm were 11(6.96%), 1-3 cm were 25 (15.82%), 3-5 cm 83(52.53%) and in more than 5 cm were 39 (24.68%) in table 3.

Table 3. Number and percentage of hydatid cysts according to sizes in liver of cattle

Size	**1>cm**	**1-3 cm**	**3-5 cm**	**5<cm**
Number	11	25	83	39
Percentage *	06.96%	15.82%	52.53%	24.68%

Figure 1. Photograph of cattle liver showing a large number of hyatid cysts with variable sizes (1-3 cm), distributed in different lobes

Figure 2. Photograph of lung sheep showing a single hydatid cyst with 4 cm dimensions

Histopathology Results

The histopathology results, all sections of lung reveled fibrous tissue reaction(capsules), necrosis, cellular reaction, and collapsed lung of tissue adjacent the wall of cyst, were as liver sections reveled fibrous tissue reaction, atrophy and necrosis of hepatocytes, cellular infiltration adjacent the cyst wall. Parasite layers (laminated and germinativm layers) in the majority of the sections observed, some were continuous and undamaged as in figure 3, retaining protoscolices, whilst others are disrupted. Other sections in both lung and liver sections, there were just residues of these layers. The thickness and number of laminations of the laminated layers varied. In liver tissue, parasitic sections (scolices) were seen in varying numbers in most sections, although this aspect was not seen in lung tissue. Cellular infiltrations could be severe, minor (scanty), or localized, however some areas had a tendency to develop granulomas with the formation of giant cells. Mononuclear cells, neutrophils were the most common types of infiltrating inflammatory cells. Fibrous tissue reaction (capsule) was moderate in sheep lung sections, while cellular reactivity was moderate in the adventitial layer and extended to the cyst's inner wall. The most common inflammatory cells type was monocyte; a wide zone of fibrous tissue reaction capsules was observed in liver section. Cellular infiltrations were generally diffuse, and inflammatory cells mainly consisted of mononuclear cells and neutrophils infiltrate as in figure 4. Many damaged, necrotic hepatocytes surround the cyst wall. In table 4 when compare between parasitic and host layers in the thickness there was significant difference in all size groups except 1-3 cm. in liver of sheep , while in lung of sheep there was significant difference in thickness of parasitic layers and host layer in all size groups .In liver of cattle there was significant difference in thickness of parasitic layer and host layer in size groups <1 cm and 3-5 cm.when using special stain(Masson's trichrome staining) revealed sever fibrosis as in figure 5 .

Figure 3. Microscopic section of infertile hydatid cyst daughter cysts in liver of sheep revealed the three layers consisting of the wall of cyst with inflammatory cells infiltration particularly in adventitial layer (H&E stain,100X)

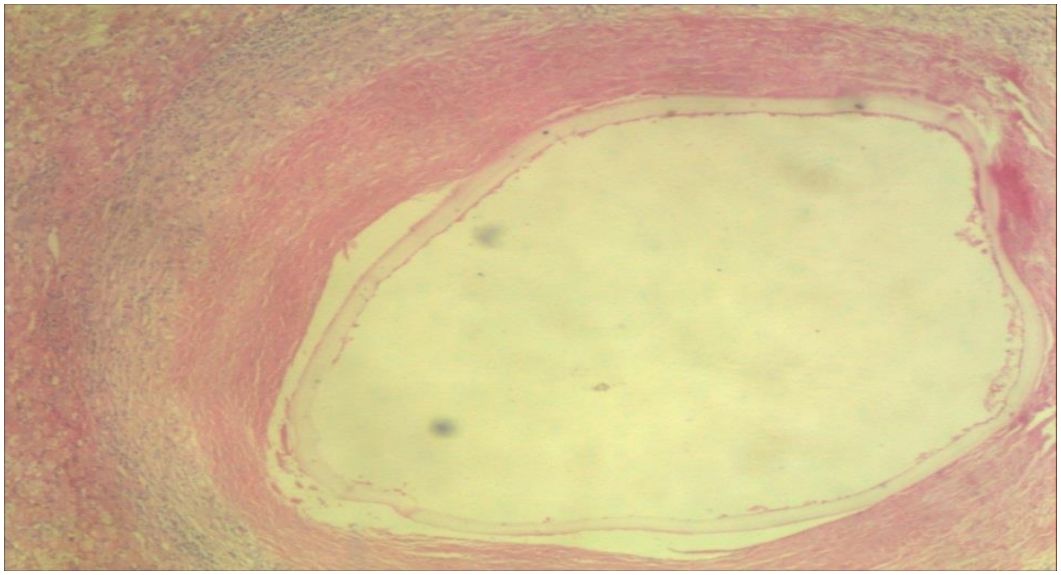

Figure 4. Microscopic section of infertile hydatid cyst in liver of sheep revealed the three layers consisting of the wall of cyst with inflammatory cells infiltration particularly in adventitial layer (H&E stain,100X)

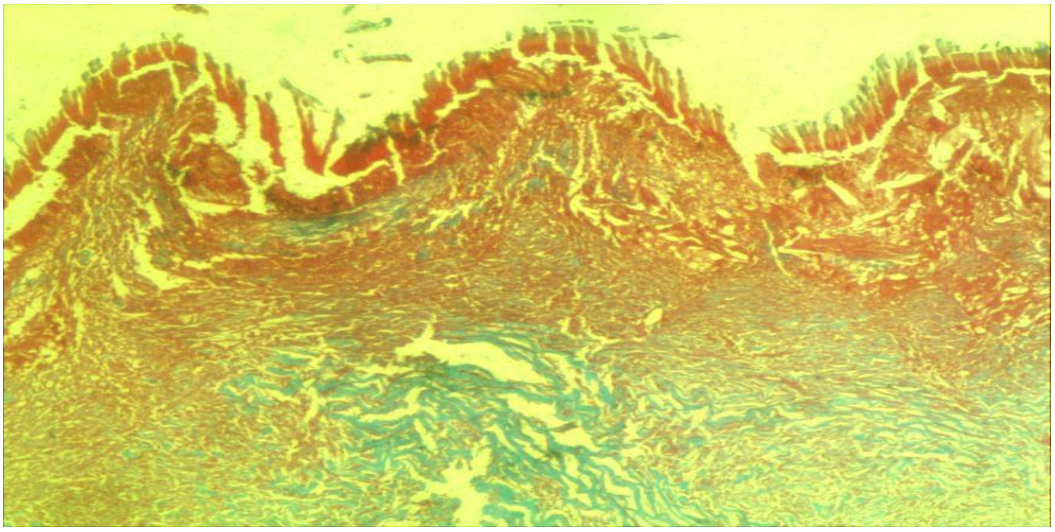

Figure 5. Microscopic section of hydatid cyst in Liver of cattle revealed diffuse fibrous deposition, replacing the hepatic tissue (black arrow) (Masson's trichrome stain 100x)

Table 4. Mean of The thickness of Parasitic Layers and Host Layer Among Size Group in Liver and Lung of Sheep and Liver of Cattle.

Animals/organ	layers	1< cm	1-3 cm	3-5 cm	5> cm
Liver /sheep	Parasitic layers	18.40*	15.44	26.10*	24.24*
	Host layer	11.80	16.12	18.04	22.31
Lung/sheep	Parasitic layers	11.62*	16.73*	16.31*	17.28*
	Host layer	17.87	20.59	21.13	21.07
Cattle/sheep	Parasitic layers	20.61*	18.51	17.61*	20.80
	Host layer	27.21	21.06	21.46	22.46

* There was significant difference in the thickness of parasitic layers and host layer in all size groups except 1-3 cm. in liver of sheep, while in lung of sheep there was significant difference in thickness of parasitic layers and host layer in all size groups. In liver of cattle there was significant difference in thickness of parasitic layer and host layer in size groups <1 cm and 3-5 cm.

Discussion

The number of cysts in the liver was higher than in lung of slaughtered sheep, consistent with that reported by [17]. This may belong to chemical, physical or biological damages as well as the structural and histological properties of the liver this observation reported by [18]. The hepatic lesions were most prominent in the livers of slaughtered cows; nearly half of the size of the hydatid cyst in sheep livers was between 1-3 cm, while in cattle it was between 3-5 cm; this was due to an anatomical feature of the animal and related to the bile duct in which blood is received by the liver along with oncospheres after having to pass through the duodenum, also due blood vessels size and size of cyst as reported by [19] and [20]. The histopathological results showed features of chronic inflammation in hepatic tissue of infested sheep and cattle by cystic echinococcosis represented by peripheral fibrous tissue enveloped the hydatid cyst, infiltration of mononuclear cells, eosinophils, neutrophils, macrophages, and fibrocytes, especially in adventitial and around the fibrous layer. Eosinophilia is a common consequence of parasitic infection consistent with that reported by [21]. Scolices observed in the liver sample of sheep in many sections, while this feature was not observed in infected lung sections. The hydatid cyst is surrounded by an adventitial layer, which is composed mostly of connective tissue and is produced by the intermediate host in reaction to the parasite). As a result of the host's immunological response to the cyst as a foreign body, the adventitial layer has varying thickness and may show some localized fibrosis this consistent with that reported by [22], [23]. Our research was done at a microscopic level the hydatid cyst lumen is filled with hydatid fluid and is surrounded by layers of parasite tissue; the germinal layer is the most innermost cellular layer and is related to an acellular layer known as the laminated layer, which is in close

contact with the adventitial layer. The germinal layer is made up of embryonic cells that are responsible for elaborating the various elements of hydatid cysts. These embryonic cells mature into buds, which then mature into protoscoleces (PSC), the parasite's infective form. The germinal layer produces the laminated layer, which is described as a specific extracellular matrix found uniquely in the *Echinococcus* genus, consistent with that reported by [24]; and [22]. In several cases, calcifications were visible as revealed in, this agreed with that reported by [25]and [26].

When the thickness of parasitic layers (germinal and laminated) was compared to the thickness of the host layer (adventitial) in the liver of sheep, it was revealed that there was a significant difference in parasitic layer and host layer thickness in all size groups except the 1-3 cm, In the lungs of sheep, there was a considerable difference in parasitic and host layer thickness in all size groups. Although there was a significant different in parasitic layer and host layers thickness in liver of cattle in size groups of 1 cm and 3-5 cm. Individual differences were linked to a variety of characteristics, including the host's age, gender, and immunological response,these results consistent with that reported by [27], as well as the presence of laminated layer is recognized with genus Echinococcosis as mentioned by [28]. In the laminate layer, which consists of organized fibrous tissue with scattered inflammatory cells, Masson's trichrome stain revealed the fibrous capsule in all of the cysts' walls, which were graded depending on staining intensity. Some sections in the liver of cattle showed diffuse fibrosis in hepatic tissue and proliferation of blood vessels in the adventitial layer of cyst, which appeared blue for stain, while germinativum cells appeared nucleated and dark in color, these also reported by ([29];[30] ;[25].

Conclusions

There is significant difference between thickness of germinativum, laminated and adventitial layers of hydatid cysts in different size groups, there was significant difference between thicknesses of parasitic layers with host layer in most size groups, no hydatid cysts recognized in lung of slaughtered cattle during the study period.

Conflict of interest

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

References:

1. A. M. Salih, D. M. Ahmed, F. H. Kakamad, R. A. Essa, A. H. Hunar, and H. M. Ali, “Primary chest wall Hydatid cyst: Review of literature with report of a new case,” *Int. J. Surg. Case Rep.*, vol. 41, pp. 404–406, 2017.
2. A. A. Mohammed and S. H. Arif, “Hydatid cyst of the parietal peritoneum,” *J. Pediatr. Surg. Case Reports*, vol. 43, pp. 80–82, 2019.
3. E. T. Tigen, B. E. Sengel, H. Bilgin, S. Ozel, and L. Mulazimoglu, “Reports of four cyst hydatid cases in different size and location,” *Asian Pacific J. Trop. Dis.*, vol. 4, no. 5, pp. 408–411, 2014.
4. Y. A. J. Fdaladdin, A. I. Alsaggaf, and M. H. Wakid, “Comparative epidemiological studies on Echinococcosis of local and imported livestock in Al-madina Al-munawwarah in Saudi Arabia,” *Egypt. J. Hosp. Med.*, vol. 50, no. 1, pp. 108–126, 2018.
5. J. Eckert *et al.*, “Chapter 3: Echinococcosis in animals: clinical aspects, diagnosis and treatment,” *WHO/OIE Man. Echinococcosis Humans Anim. A Public Heal. Probl. Glob. Concern. WHO/OIE, Paris, Fr.*, 2001.

6. M. Gerazounis, K. Athanassiadi, E. Metaxas, M. Athanassiou, and N. Kalantzi, "Bronchobiliary fistulae due to echinococcosis," *Eur. J. cardio-thoracic Surg.*, vol. 22, no. 2, pp. 306–308, 2002.
7. M. Daali, Y. Fakir, R. Hssaida, A. Hajji, and A. Hda, "Hydatid cysts of the liver opening in the biliary tract. Report of 64 cases," in *Annales de chirurgie*, 2001, vol. 126, no. 3, pp. 242–245.
8. K. A. Faddladdeen, "Comparative Liver Histopathological and Histochemical Studies in Hydatid Cyst Disease with Mean Optical Transparency," *Pharmacophores*, vol. 10, no. 2, pp. 51–62, 2019.
9. M. A. Ciftçioğlu, M. I. Yildirgan, M. N. Akçay, A. Reis, M. Safali, and E. Aktaş, "Fine needle aspiration biopsy in hepatic Echinococcus multilocularis.," *Acta Cytol.*, vol. 41, no. 3, pp. 649–652, 1997.
10. L. H. Mahmoud and M. F. el-Garhy, "A histochemical study on the hydatid cyst and electron microscopy of the hydatid sand of Echinococcus granulosus.," *J. Egypt. Soc. Parasitol.*, vol. 32, no. 2, pp. 647–656, 2002.
11. A. Stevens, J. S. Lowe, B. Young, P. R. Wheater, and H. G. Burkitt, *Wheater's basic histopathology: a colour atlas and text*. Churchill Livingstone, 2002.
12. A. A. Rashed, H. M. Omer, M. A. Fouad, and A. M. Al Shareef, "The effect of severe cystic hydatidosis on the liver of a Najdi sheep with special reference to the cyst histology and histochemistry.," *J. Egypt. Soc. Parasitol.*, vol. 34, no. 1, pp. 297–304, 2004.
13. [13] H. H. Kassem and N. K. Gdoura, "Hydatidosis in camels (Camelus dromedarius) slaughtered at Sirt Abattoir, Libya.," *J. Egypt. Soc. Parasitol.*, vol. 36, no. 2 Suppl, p. 1, 2006.
14. F. M. Haridy, S. A. Holw, A. A. Hassan, and T. A. Morsy, "Cystic hydatidosis: a zoonotic silent health problem.," *J. Egypt. Soc. Parasitol.*, vol. 38, no. 2, pp. 635–644, 2008.
15. K. Abdul and J. Faddladdeen, "Pharmacophore COMPARATIVE LIVER HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIES IN HYDATID CYST DISEASE WITH MEAN OPTICAL TRANSPARENCY," vol. 10, no. 2, pp. 51–62, 2019.
16. A. M. Brown, "A new software for carrying out one-way ANOVA post hoc tests," *Comput. Methods Programs Biomed.*, vol. 79, no. 1, pp. 89–95, 2005.
17. S. E. A. Ibrahim, "Pathological, histochemical and immunohistochemical studies of lungs and livers of cattle and sheep infected with hydatid disease," 2014.
18. F. Ahmedullah *et al.*, "Pathological investigation of liver of the slaughtered buffaloes in Barisal district," *Bangladesh J. Vet. Med.*, pp. 81–85, 2007.
19. N. K. Thapa, M. T. Armua-Fernandez, D. Kinzang, R. B. Gurung, P. Wangdi, and P. Deplazes, "Detection of Echinococcus granulosus and Echinococcus ortleppi in Bhutan," *Parasitol. Int.*, vol. 66, no. 2, pp. 139–141, 2017.
20. B. Paoletti *et al.*, "Epidemiological survey on cystic echinococcosis in wild boar from Central Italy," *Parasitol. Res.*, vol. 118, no. 1, pp. 43–46, 2019.
21. A. Sevimli, F. K. Sevimli, E. Şeker, A. Ulucan, and H. H. Demirel, "Acute-phase responses in cattle infected with

hydatid cysts and microbial agents," *J. Helminthol.*, vol. 89, no. 4, pp. 471–479, 2015.

22. M. A. da Silva, "Hydatid cyst/cystic echinococcosis: anatomical and surgical nomenclature and method to quantify the cyst content solidification," *Chin. Med. J. (Engl).*, vol. 124, no. 18, pp. 2806–2812, 2011.
23. T. Sakamoto and P. A. Cabrera, "Immunohistochemical observations on cellular response in unilocular hydatid lesions and lymph nodes of cattle," *Acta Trop.*, vol. 85, no. 2, pp. 271–279, 2003.
24. A. Lucio-Forster, J. K. Griffiths, V. A. Cama, L. Xiao, and D. D. Bowman, "Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats," *Trends Parasitol.*, vol. 26, no. 4, pp. 174–179, 2010.
25. T. S. Barnes, L. A. Hinds, D. J. Jenkins, H. Bielefeldt-Ohmann, M. W. Lightowlers, and G. T. Coleman, "Comparative pathology of pulmonary hydatid cysts in macropods and sheep," *J. Comp. Pathol.*, vol. 144, no. 2–3, pp. 113–122, 2011.
26. A. B. Beigh *et al.*, "Gross and histopathological alterations associated with cystic echinococcosis in small ruminants," *J. Parasit. Dis.*, vol. 41, doi: 10.1007/s12639-017-0929-z.
27. W. Zhang, J. Li, and D. P. McManus, "Concepts in immunology and diagnosis of hydatid disease," *Clin. Microbiol. Rev.*, vol. 16, no. 1, pp. 18–36, 2003.
28. D. D. Heath *et al.*, "Vaccination of bovines against Echinococcus granulosus (cystic echinococcosis)," *Vaccine*, vol. 30, no. 20, pp. 3076–3081, 2012.
29. A. A. Rashed, H. M. Omer, M. A. H. Fouad, and A. M. F. Al Shareef, "The effect of severe cystic hydatidosis on the liver of a Najdi sheep with special reference to the cyst histology and histochemistry.," *J. Egypt. Soc. Parasitol.*, vol. 34, no. 1, pp. 297–304, 2004.
30. B. B. Singh, • R Sharma, • J K Sharma, • V Mahajan, and J. P. S. Gill, "Histopathological changes associated with E. granulosus echinococcosis in food producing animals in Punjab (India)", doi: 10.1007/s12639-014-0622-4.